INSTITUTE OF INDUSTRIAL RESEARCH

MATERIALS ENGINEERING LABORATORY

Syracuse University

Syracuse 10, New York

From: Dr. W. R. Boss

To: Chief of Naval Research
Department of the Navy

Via: Physiology Branch (Code 441)

Date: January 26, 1954
For period July 1 - December 31, 1953
Addendum to Last Semiannual Progress
Report of January 1, 1953 to June
30, 1953

From: Dr. W. R. Boss

To: Chief of Naval Research
Department of the Navy
Washington 25, D. C.

Via: Physiology Branch (Code 441)

Subject: Annual Progress Report (NR 115-096)
Addendum to report of July 31, 1953

I. Objectives of the Problem

To study the induced abnormalities in the internal distribution of body fluids and renal damage resulting from varying dosages of x-rays.

II. Progress

A. Materials and Methods

(1) Radiation procedures

X-irradiation generated at 140k.v.p. at 7ma (HVL = 4.5 mm.Al.), by a Westinghouse unit with a Machlett tube was employed. Radiation was administered in divided doses, 50% of the total dose given dorsally and 50% ventrally. Dose measurements were made prior to and following the exposure of each group of animals with a Victoreen condenser r meter. Dose rates varied between 28.0 and 34.0 r per minute at an anode distance of 35 centimeters. The animals were irradiated in a triangular aluminum cage which contained three compartments with perforations on the sides for ventilation. Control animals were sham irradiated and were placed in the aluminum cage for as long a period of time as the irradiated animals.

(2) Experimental animals

Mature hooded female rats of the Syracuse strain weighing between 190 - 220 grams were used in all experiments. Animals were selected on a basis of growth curves in order to obtain healthy and infection free animals. A total of 84 rats were used. When the animals were not in metabolism cages they were housed in individual rat cages.

(3) Experiments conducted

(a) 500r Irradiated Rats And Pair-fed Controls

Twenty-four rats weighing approximately 210 grams were divided into 2 groups of 12. The animals were then paired according to body weight. One group received 500r total body irradiation while the control group was sham irradiated. Following exposure to the x-rays, the rats were placed in individual metabolism cages for an 8 hour period daily for 28 days to collect urine. While not in the metabolism cages the rats were placed in separate cages where they were allowed free access to food and water. The food intake of each irradiated rat was measured daily, and its pair-fed twin received an equivalent amount of food the following day.

The animals were weighed daily prior to the start of the urine collection period and urine volumes were recorded at the end of each 8 hour period. Both the irradiated and control animals were allowed water *ad libitum* while in the metabolism cages. Urine was collected in 50cc. graduated cylinders. Paraffinized wire-mesh screens, placed over the collection funnels, prevented the contamination of urine samples with feces. The metabolism cages, screens, and collection funnels were washed down daily with distilled water. An aliquot of each urine sample was then analysed in the flame photometer for sodium and potassium.

When one of the animals died during the course of the experiment, the adrenals were immediately removed and weighed. The pair-fed twin of such an animal was sacrificed and an autopsy performed the following day. On day 28 the surviving animals were sacrificed and the adrenals removed and weighed.

(b) 1000r Irradiated Rats And Pair-fed Controls

Twelve rats weighing approximately 200 grams were divided into 2 groups of 6. The experimental procedure employed was the same as that in experiment (a), with the exception that the irradiated group received 1000r. Since this dose is 100% lethal, the experiment continued until the death of the last animal.

(c) 1000r Fasted Irradiated Rats and Fasted Controls

Twelve rats weighing approximately 215 grams were divided into 2 groups of 6. One group received 1000r total body irradiation while the second group was sham irradiated. Both groups were placed in metabolism cages immediately following exposure where they remained for 96 hours, at which time the experiment was terminated. Neither group had access to food but were allowed water *ad libitum*. During this period, body weight, water consumption, urine volumes, and urinary Na and K values were determined daily. Adrenal weights, both wet and dry, were taken at the end of the experiment. Dry weights were obtained by placing the adrenals in an oven at 110°C and drying to constant weight.

(d) Serum Na And K Values Following 1000r

Thirty-six animals were used in this study. Electrolyte values were determined at 2, 24, 48, 72, and 96 hours post-irradiation. Blood for analysis was drawn under anaesthesia by direct heart puncture. Adrenal weights of all animals were recorded.

B. Results

Experiment a) 500r Irradiated Rats And Pair-fed Controls

Body weights prior to irradiation and at the time of death or sacrifice are recorded in Table I. Daily changes in body weight as well as food consumption are presented graphically in Figure I. A steady decline in body weight following 500r irradiation was observed reaching the lowest points at 3 and 6 days post-irradiation. Weight loss in the pair-fed controls followed a similar pattern but was never as great

as that demonstrated by the irradiated group. A second and much less severe period of weight depression occurred in both groups from day 8 through day 20. Both groups exhibited a steady increase in body weight for the remainder of the test period.

Daily values for urinary Na and K (total m.Eq.) are shown in Figures 2 and 3 respectively. Two periods of increased Na excretion occurred in the irradiated group. The first was seen from days 6 - 10 and the second on days 21 through 24. The elevated Na values can not be due to an increased urine output for the urine volumes of the irradiated rats on days 6 - 10 and 21 - 24 were actually lower than the controls.

Following an initial rise in urinary K in the irradiated group on the first day post-irradiation, daily K values generally paralleled each other. An unexplainable sharp rise in urine K was observed on day 20 in the control group and a similar situation was seen in the irradiated group on day 22. Daily urine volumes for both groups are presented in Figure 4.

Adrenal weights, expressed as mg./100 grams body weight are listed in Table I. There can be little if any doubt that the adrenals are called upon for increased hormonal production following irradiation. Attention is called to the two animals that died following 500r irradiation (Experimentals #3 and #6). The adrenals of surviving irradiated rats, although almost always larger than their pair-fed twins, were not nearly as large as the above two animals illustrating the additional stress that must be placed on the adrenals when a rat is dying from the effects of 500r total body irradiation.

Experiment b) 1000r Irradiated Rats And Pair-fed Controls

Body weights prior to irradiation and at the time of death or sacrifice, daily weight changes, food consumption and adrenal weights of both groups are recorded in Table II. All of the irradiated rats were dead by the end of five days. The daily weight loss in the irradiated animal was always greater than in the pair-fed twin. Irradiated rats at this dose level decrease their food intake sharply with complete anorexia on days 3 and 4. Adrenal hypertrophy, Table II, in the irradiated group was very evident at this dose level. The adrenals of the control animals are also larger than normal. This is probably due to the stress of decreased food intake.

Electrolyte values for urinary Na and K are presented graphically in Figures 5 and 6 respectively. Urine values for both groups are also listed in order that electrolyte excretion can be compared with urine output. Very little difference is evident in urinary Na values for the irradiated and control groups. At first sight this may seem odd since Na loss appeared to be prominent in the 500r irradiated rats. However, the changes in metabolism following 1000r are undoubtedly much more severe and may be quite different than those changes occurring in 500r irradiated rats. Urinary K in the irradiated group is significantly higher on the first day post-irradiation and reaches its lowest point on day 5.

Experiment c) 1000r Fasted Irradiated Rats And Fasted Controls

Since weight loss in the irradiated rats was greater than the pair-fed controls in both experiment (a) and (b), it was decided to check the weight changes in fasted irradiated and fasted non-irradiated rats. It has been reported that weight loss in such animals is essentially the same (Nims and Sutton, Am. J. Physiol. Vol. 171:17-21, 1952). A possible sex difference may exist for they used males while females were employed in this study. The changes in body weight of fasted irradiated and fasted controls are recorded in Table III. It can readily be seen that weight loss in the former is much greater than the latter. Consequently the loss of weight following irradiation does not appear to be solely due to the anorexia the animals exhibit.

Electrolyte values for urinary Na and K are presented graphically in Figures 7 and 8 respectively. Urine volumes for both groups are also listed beneath the graphs. As in experiment (b) little change in Na loss takes place. This is true on day I even when the irradiated rats excreted more than twice as much urine as the controls. Urinary K loss appears to be prominent following 1000r total body irradiation and is significantly higher than that of the controls on day 1 and 3. Adrenal weights recorded in Table III reaffirm the marked adrenal hypertrophy observed in the irradiated rats of experiments (a) and (b).

Experiment d) Serum Na And K Values Following 1000r

Electrolyte values for serum are recorded in Table IV. The serum potassium remained essentially unchanged while serum Na showed a significant increase at only one time, i.e. 2 hours post-irradiation. A progressive increase in adrenal weights (Table IV) was observed, even as early as 2 hours post-irradiation.

C. Conclusions:

The above data suggest the following conclusions:

1. The weight loss in rats receiving 500r and 1000r total body irradiation can not be due to anorexia alone.

2. Although the loss of Na in rats receiving 500r total body irradiation is significantly greater than the pair-fed controls this does not appear to figure significantly in those animals receiving 1000r.

3. Potassium loss is increased significantly in the irradiated rats on the first day post-irradiation in both 500r and 1000r groups, although it is greater in the latter groups.

4. Marked adrenal hypertrophy was observed in all experiments. An increase in adrenal weight was recorded as early as 2 hours post-irradiation.

5. No change was found in serum K following 1000r total body irradiation while serum Na was temporarily elevated significantly at 2 hours post-irradiation.

D. Other Information

1. Publications.

Paper to be presented at Federation meetings this April.

2. Graduate students on contract.

a) One (1) full time research assistant.

b) Two (2) technicians on hourly basis.

3. University furnishes the following support.

a) One (1) full time animal caretaker.

b) Secretarial help.

4. No major difficulties encountered.

5. Immediate plans for the future.

a) Repetition of experiments reported.

b) 500r fasted rats and fasted non-irradiated controls.

TABLE I

Body weights and adrenal weights in 500r and pair-fed controls

Animal	Original Weight	Final Weight	Remarks	Adrenal Weight mg/100 gms of body weight
Experimental #1	212	217	-------	22.58
Control	210	218	-------	24.31
Experimental #2	204	206	-------	21.84
Control	205	204	-------	21.57
Experimental #3	214	157	Dead on day 20	64.96
Control	215	176	Sacrificed on day 20	23.86
Experimental #4	205	200	-------	31.00
Control	200	206	-------	21.36
Experimental #5	203	205	-------	28.78
Control	200	209	-------	22.01
Experimental #6	200	161	Dead on day 17	62.11
Control	203	166	Sacrificed on day 17	33.73
Experimental #7	200	206	-------	24.75
Control	202	205	-------	21.95
Experimental #8	202	206	-------	22.81
Control	200	208	-------	19.23
Experimental #9	210	218	-------	28.89
Control	208	222	-------	18.47
Experimental #10	200	208	-------	25.48
Control	205	208	-------	23.07
Experimental #11	210	214	-------	21.49
Control	208	213	-------	21.59
Experimental #12	200	198	-------	25.75
Control	203	210	-------	19.52

TABLE II

Body weights and adrenal weights in 1000r irradiated rats and pair-fed controls

Wgt. loss in grams/day

Animal	Body Wgt. Pre-x-ray	1	2	3	4	5	Body Wgt. at death	Adrenals Wgt.* mg/100 gm
Experimental #1	196	-13	-22	-35	-52	---	144	55.55
Control	201	- 6	-16	-23	-39	---	162	31.48
Experimental #2	206	-19	-15	-24	-25	-36	170	45.29
Control	204	- 9	- 4	-18	-23	-25	179	31.84
Experimental #3	190	-12	-13	-23	-30	-42	148	73.65
Control	190	- 6	-10	-16	-21	-25	165	40.61
Experimental #4	205	- 7	-13	-26	-32	-45	160	71.25
Control	212	0	- 5	-17	-22	-29	183	32.24
Experimental #5	197	-11	-18	-29	-42	---	155	46.45
Control	196	- 6	-11	-22	-29	---	167	34.73
Experimental #6	196	- 9	-13	-23	-30	-43	153	54.25
Control	201	- 6	-11	-19	-26	-30	171	31.58
Av. Experimental	198.3	-11.8	-15.6	-26.6	-35.1	-41.5	155.0	57.74
Av. Control	200.6	- 5.5	- 9.5	-19.1	-26.6	-27.2	171.1	33.74
Mean Food Intake in Grams	---	5.0	4.8	0	0	1.8	---	---

*Adrenal weights based on body weight at time of death

TABLE III

Body weights and adrenal weights in 1000r fasted irradiated rats and fasted controls

Animal	Body weight Pre-x-ray	Wgt. loss in grams/day: 1	2	3	4	Body weight at death	mg/100gm
Experimental 1	209	-20	-30	-50	-55	154	59.74
Control	210	-17	-26	-34	-43	167	26.94
Experimental 2	229	-21	-32	-49	-61	168	44.64
Control	228	-13	-22	-32	-39	189	25.92
Experimental 3	221	-22	-34	-46	-56	165	45.46
Control	224	-14	-23	-27	-36	188	29.25
Experimental 4	207	-22	-30	-39	-47	160	41.87
Control	200	-17	-25	-35	-40	160	30.63
Experimental 5	210	-21	-28	-39	-44	166	45.18
Control	211	-14	-21	-27	-31	180	22.22
Experimental 6	215	-18	-30	-42	-52	163	40.49
Control	211	-17	-22	-31	-38	173	34.68
Experimental Average	215	-20.6	-30.6	-44.1	-52.5	162	46.23
Control Average	214	-15.3	-23.1	-31.0	-37.8	176	28.27

Table IV.

Serum Na and serum K (m.Eq./L) following 1000r

Group	Controls	Post-irradiation 2 hours	24 hours	48 hours	72 hours	96 hours
Serum Na	146.9	153.6	145.7	146.5	141.1	143.9
Serum K	6.5	7.5	6.0	6.6	5.9	6.2
# of animals	6	6	6	6	6	6
Adrenal weights mg/100 grams body weight	23.56	24.83	29.89	30.18	40.39	51.09

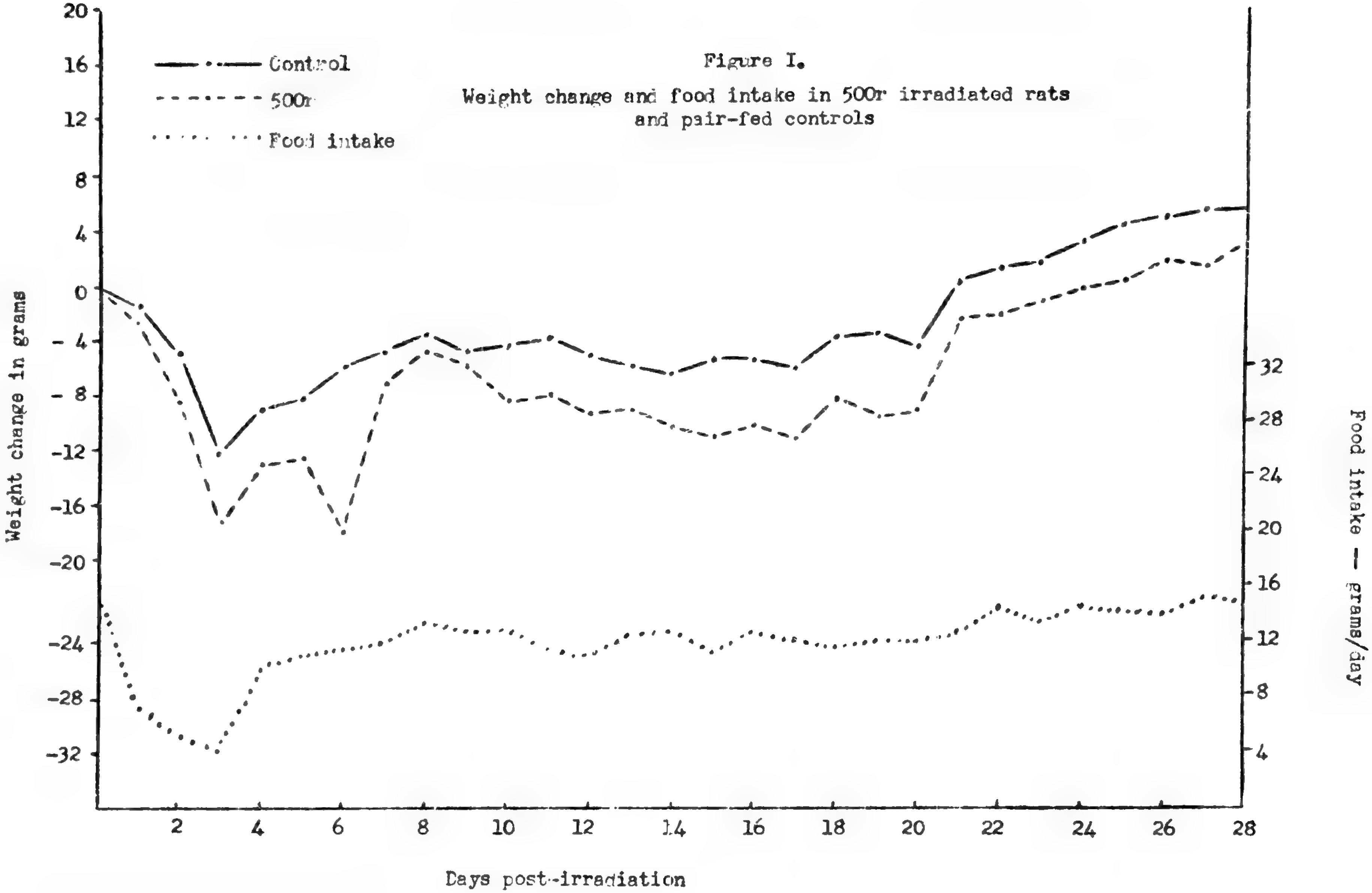


Figure I.

Weight change and food intake in 500r irradiated rats and pair-fed controls

Figure II.

Daily (8 hours) Na excretion in 500r irradiated rats and pair-fed controls

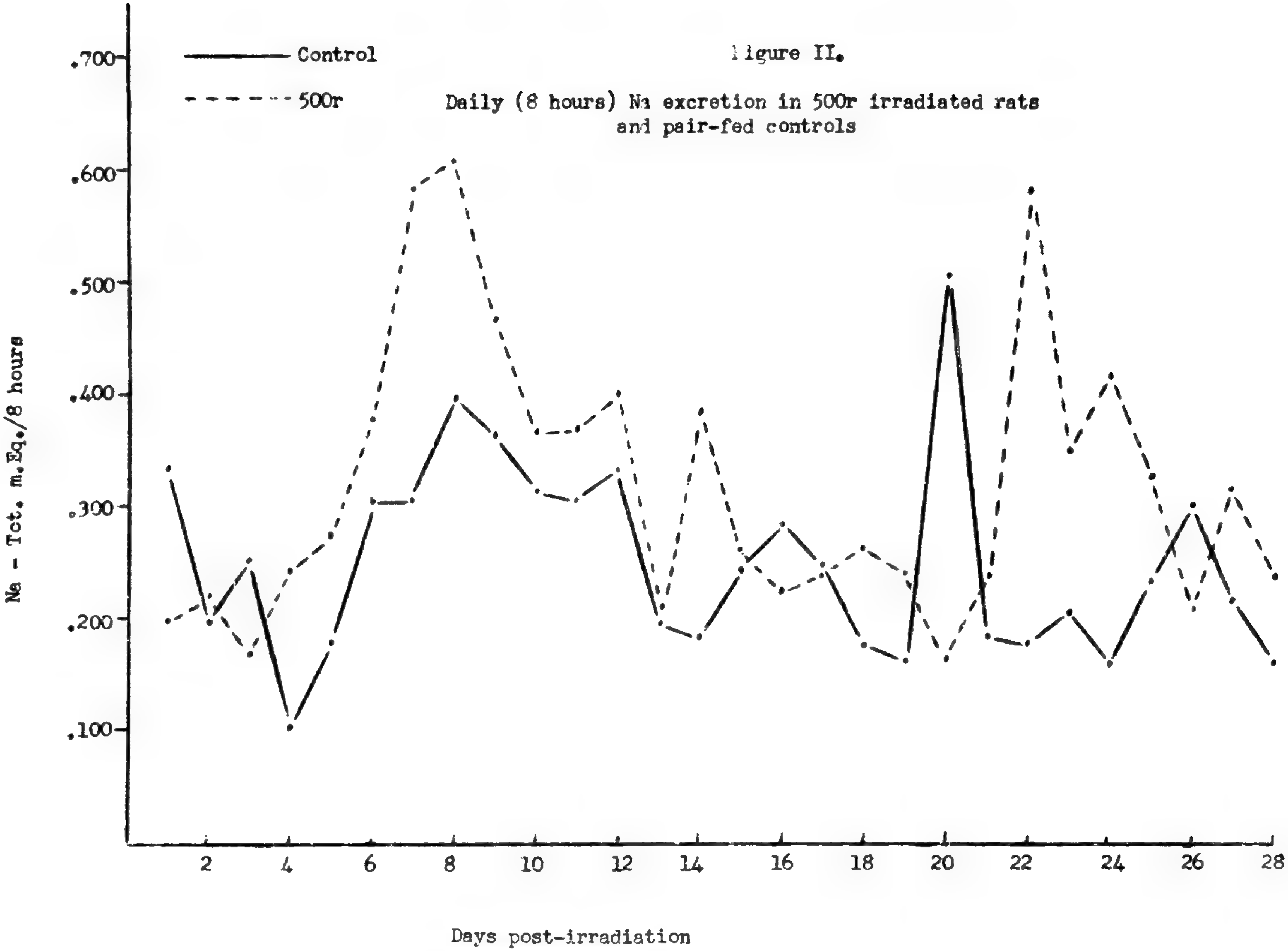


Figure III

Daily (8 hours) K excretion in 500r irradiated rats and pair-fed controls

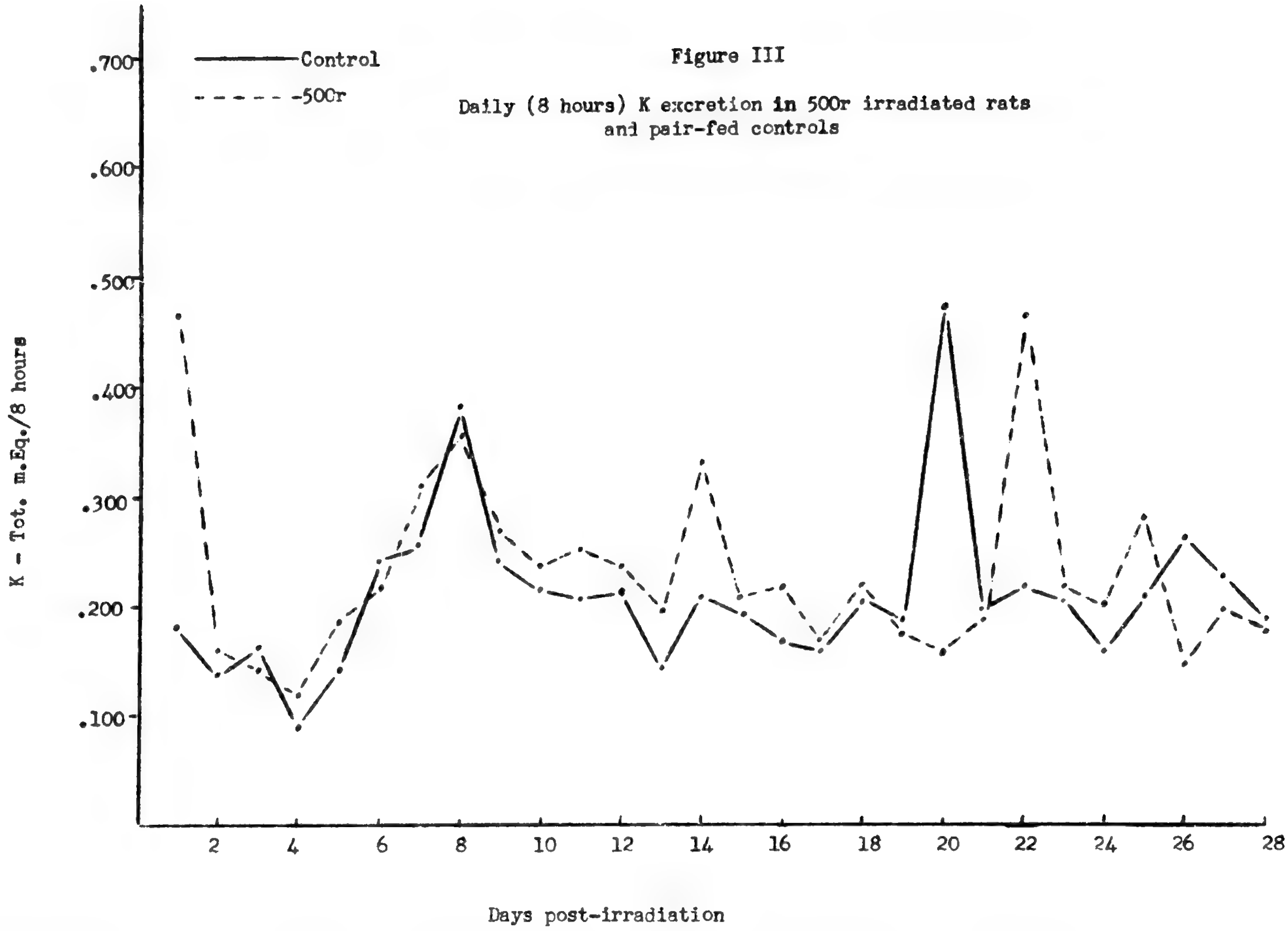
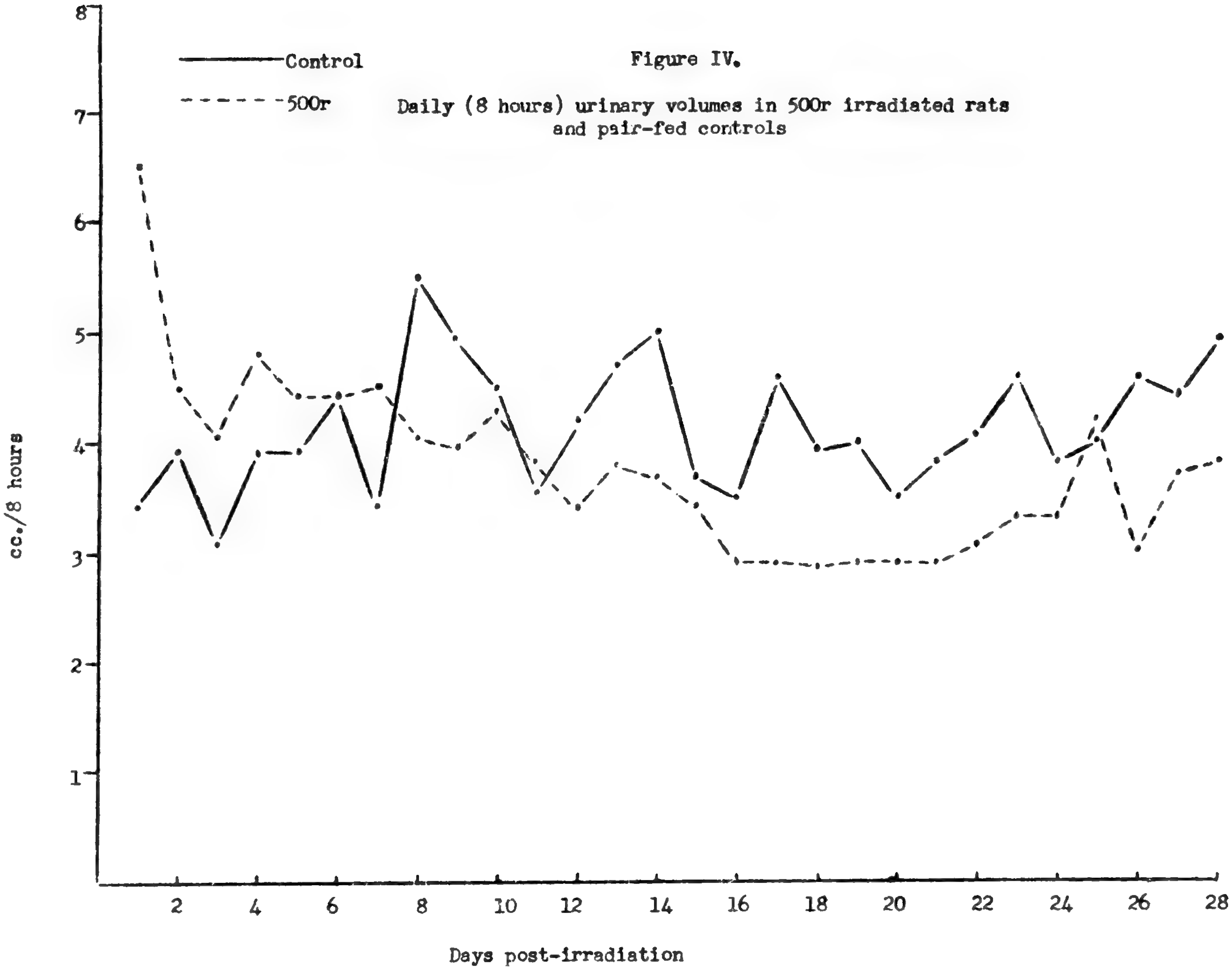


Figure IV.

Daily (8 hours) urinary volumes in 500r irradiated rats
and pair-fed controls



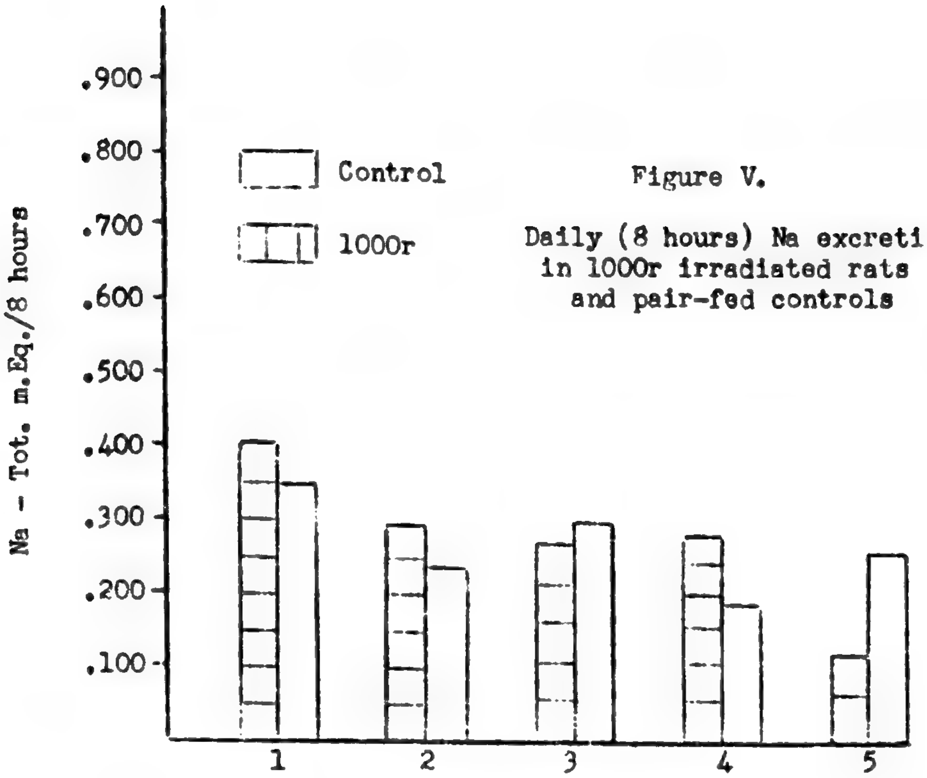


Figure V.

Daily (8 hours) Na excretion in 1000r irradiated rats and pair-fed controls

Days post-irradiation

Urine (cc's)	Irradiated	10.5	5.4	2.8	1.4	4.2
	Control	2.3	2.4	2.4	2.7	3.9

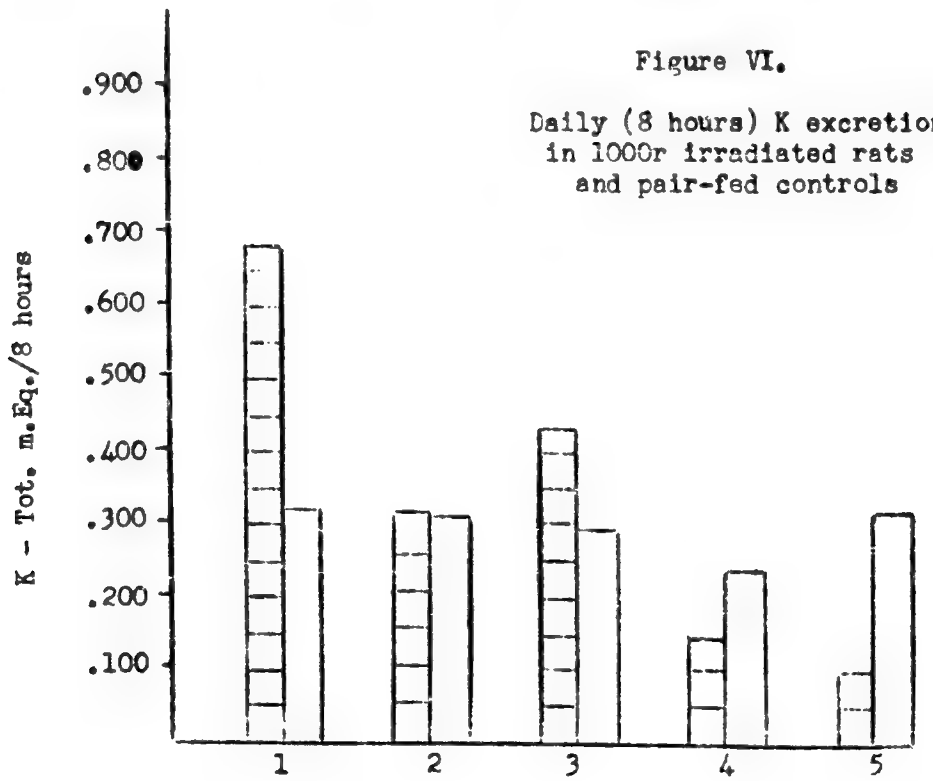


Figure VI.

Daily (8 hours) K excretion in 1000r irradiated rats and pair-fed controls

Days post-irradiation

Figure VII.

Urinary Na in 1000r fasted rats and fasted non-irradiated controls

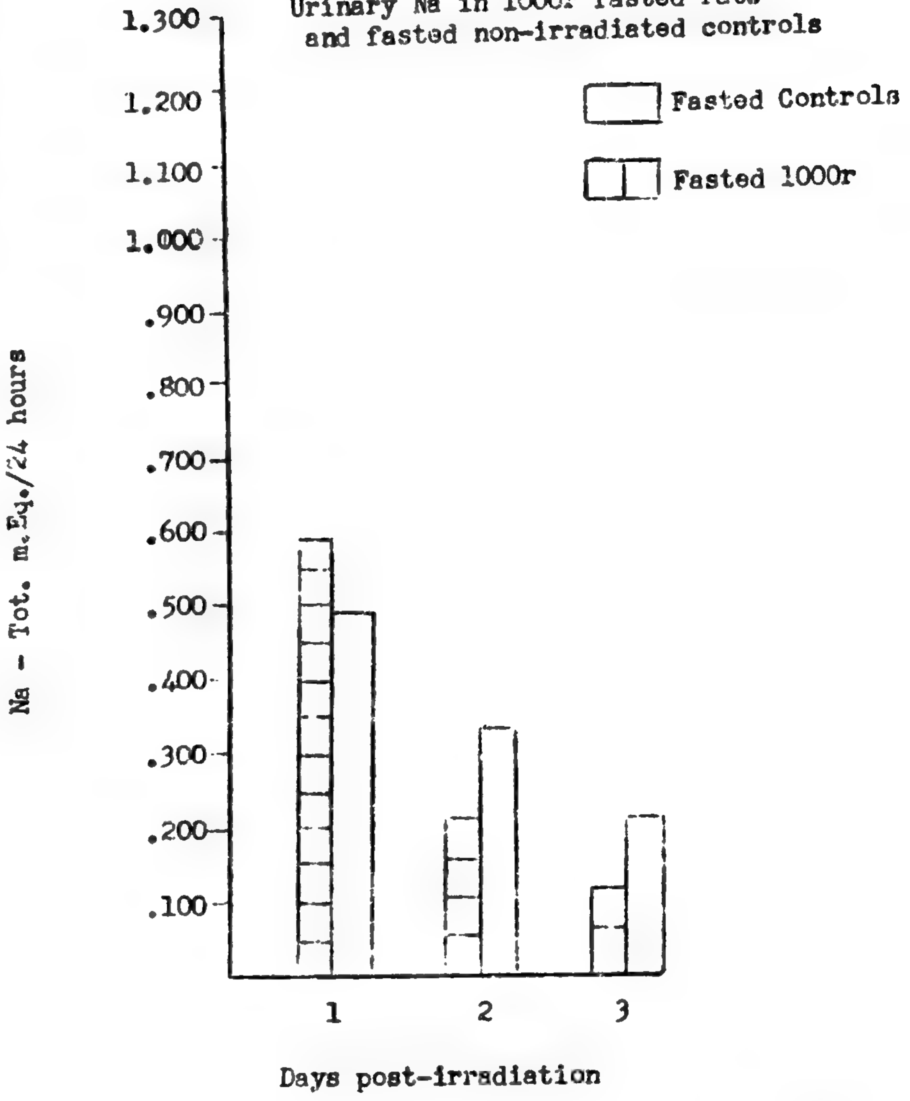
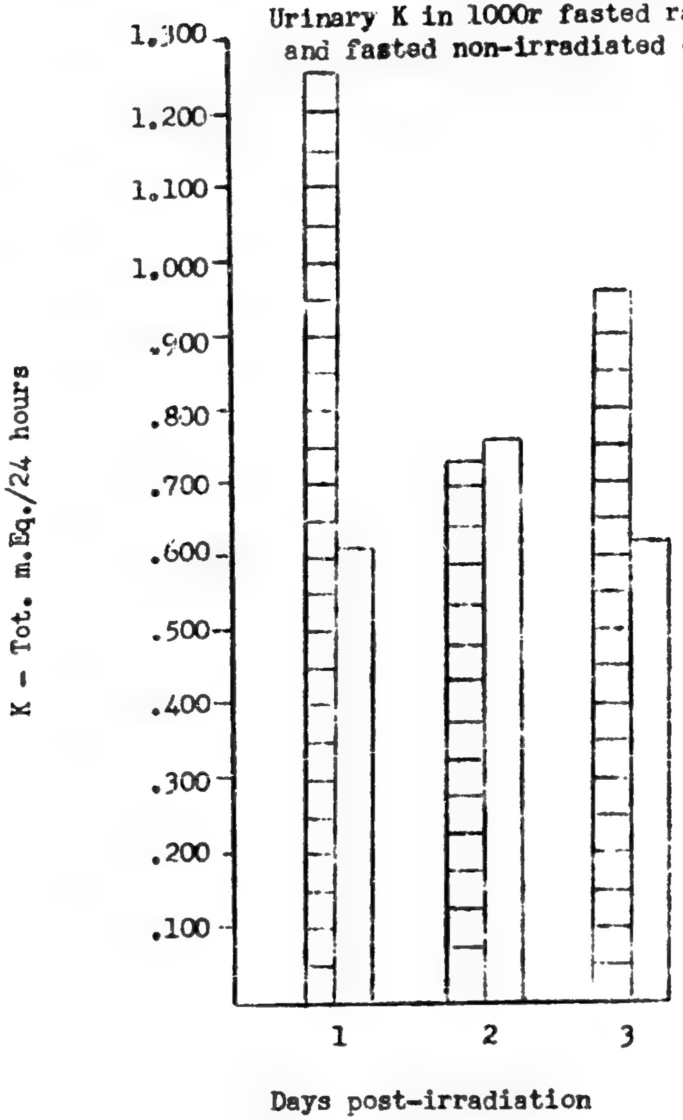


Figure VIII.

Urinary K in 1000r fasted rats and fasted non-irradiated controls



	DAY	I	II	III
Urine volume (cc's/24 hours)	Irradiated	35.2	7.7	4.5
	Control	14.2	10.0	10.7

From: Drs. W.R. Boss and H.J. Evans
Department of Zoology, Syracuse University

To: Chief of Naval Research
Department of the Navy

Via: Physiology Branch (Code 441)

From: Drs. W. R. Boss and H. J. Evans July 31, 1953

To: Chief of Naval Research
Department of the Navy
Washington 25, D. C.

Via: Physiology Branch (Code 441)

Subject: Semiannual Progress Report (NR 115-096)

1. <u>Objectives of the Problem</u>

To study the induced abnormalities in the internal distribution of body fluids and renal damage resulting from varying dosages of x-rays.

2. <u>Progress</u>

A. Materials and Methods

(1) Non-pregnant intact rats

Mature hooded female rats of the Syracuse strain weighing approximately 220 grams were used in all experiments. They were fasted for 18 hours prior to x-radiation but were allowed water. Rats received total body x-radiation at one of three dose levels, 500r, 600r or 700r (± 5%). Unless otherwise stated, the data presented here will refer to rats that received 600r or 700r. The radiation factors were 140 KVP, 7 ma and a sufficient thickness of aluminum, which included the cover of the cage, was used to produce a radiation quality corresponding to a HVL of 4.5 mm. of aluminum. Intensity of the beam was approximately 30r per minute in air at an anode distance of 35 cm. In order to equalize the tissue dose throughout the whole body, half of the total dose was delivered to the dorsal and ventral sides of the rat. Dose measurements were made at the center of each cell of the cage prior to the exposure with a Victoreen condenser r meter. The aluminum cage was triangular and contained three compartments with perforations on the sides for ventilation. The top and bottom were 0.83 mm. thick, while the sides were 3.15 mm. thick. The cage was placed on a Masonite shelf for the dorsal exposure and to insure uniform backscatter for both ventral and dorsal exposures a corresponding piece of Masonite of similar thickness was placed on top of the cage while the radiation was entering the ventral portion of the rat.

Diuretic tests were conducted after x-radiation as follows: <u>day</u> 0 (immediately after radiation), 1, 7, 14 and 30. In the 500r group tests were only made 30 days after radiation. Prior to the diuretic test the animals were fasted 18 hours. At the end of the fast, one dose of distilled water amounting to 4 ml. per 100 sq. cm. of body surface was

warmed to 100°F. and administered by stomach tube. The dose for a 220 gram rat was 13 ml. with the body surface calculated according to the formula of Benedict (1938). The rats were then placed in individual metabolism cages and urine excretion recorded at 30 minute intervals for a period of 180 minutes. Electrolyte determinations were made of the urine collected for the periods of 0-90 minutes (first collection period) and 90-180 minutes (second or terminal collection period). At the terminal collection period the rats were anesthetized with ether and blood samples from the tail were obtained for hematocrit determinations. Four to five ml. of blood for electrolyte analysis was then drawn under mineral oil by a direct heart puncture and immediately centrifuged for 10 minutes. Sodium and potassium values[1] were determined by an internal standard flame photometer. All animals were weighed prior to and every day after radiation. Blood pressure determinations were taken every second day with a Metro photoelectric tensometer.

The following data for each series of non-pregnant rats is as follows:

Diuretic rates (percentage of administered water excreted), electrolyte analysis of serum and of the 90 and 180 minute urine samples, systolic blood pressures, and hematocrits.

Series 1 Controls . . . . 12 females

Series 2 Day 0. 24 females
12 animals x-radiated at 600r
12 animals x-radiated at 700r

Series 3 Day 1. 24 females
12 animals x-radiated at 600r
12 animals x-radiated at 700r

Series 4 Day 7. 40 females
21 animals x-radiated at 600r
19 animals x-radiated at 700r

Series 5 Day 14 . . . . 45 females
18 animals x-radiated at 600r
27 animals x-radiated at 700r

Series 6 Day 30 . . . . 55 females
18 animals x-radiated at 500r
20 animals x-radiated at 600r
17 animals x-radiated at 700r

(2) Pregnant intact rats

Pregnant females were exposed to x-rays for a total dosage of

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[1] The term "concentration" of the electrolytes indicates m Eq/l. while "excretion" or "loss" refers to total m Eq.

250r at 12 days of pregnancy. These rats were then subjected to one of the tests described below. Non-pregnant, x-rayed rats served as controls.

A diuretic run was conducted as follows: Animals were starved for 12 hours prior to the start of the run. Distilled water warmed to body temperature was given via stomach tube. The dose was calculated on the basis of 3 ml. for every 100 sq. cm. of body surface and two doses were given one hour apart. Urine was collected for three hours after the second water dose. At hourly intervals urine samples were taken for Na and K analysis. Since the bladders were emptied by suprapubic pressure prior to the first water dose, the urine put out between the first and second doses was added to that excreted during the hour following administration of the second water dose. Three hours after the second water dose the animals were sacrificed, hematocrits were taken and heart blood drawn for Na and K determinations on the serum. In the pregnant animals the fetuses were examined for gross defects which might have been induced by x-rays.

Since the diuretic run described above precludes the subsequent use of the same animals, a second type of urine collection method was utilized. Experimental and control animals were placed in metabolism cages for four hours each day from the day of radiation to the day of delivery. This enabled us to follow any urine electrolyte changes on a day to day basis for each animal. Four hours was used as the collection period, for no satisfactory method of feeding the animals in the metabolism cages could be devised. Of the several methods tried, none prevented food particles from falling into the funnel. The collections were made between 1000 and 1400 each day on the theory that this would be about the middle four hours of the daylight period and the metabolic activity might be most easily compared over a ten day period.

B. Results

(1) Non-pregnant intact rats (3 tables and 4 graphs)

(a) Diuretic Rates

There was a marked increase in the diuretic rates in all of the x-radiated animals during the first 120 minutes of the collection period with the exception of the two day 14 series (Table 1). The diuretic rates of the day 1 and 30 (600r and 700r) was consistently higher than the controls and other experimental series from the beginning of the water test. However the total percentage of water excreted in 180 minutes by the experimental rats was not significantly different from that of the controls.

(b) Serum Electrolytes

Table 2 shows that the serum sodium levels of all radiated animals were increased, but only, in the day 30 series was this increase significant. The serum potassium concentrations (Table 3) of

animals that received 600r or 700r were depressed on days 7 and 14 but were within the normal range in the other three series. There was no consistent pattern as trend in the serum electrolyte concentrations of the experimental groups.

(c) Urine Electrolytes

Sodium

The rate of sodium excretion of the control and day 0 series was greatest during the second 90 minute or terminal collection period (Table 2). The day 1, 7, 14 and 30 series, given 700r, showed the greatest sodium loss during the first 90 minutes. However, there was no specific trend in the series that received the lesser dose.

The sum of the total m Eq. of the first and second collection periods was greater than the controls at the three radiation dose levels in all the series with the exception of day 14 (Graph 1).

In the series receiving 600r the sum total loss[2] of the sodium-ion during the 180 minute collecting period progressively increased as the time after radiation increased with the exception of the day 14 Series (Graph 1). The animals that received the higher dose followed a similar pattern, namely, sodium loss increases with post-radiation time. The greatest single sodium loss during any collection period was observed during the terminal period of day 30, 500r group. This sum total loss of the ion for the two 90 minute periods was the greatest of all the experimental series. Indications are that if there had been a 100 per cent survival in the other two day 30 series, 600r and 700r, a similar high sodium excretion would have been observed.

Potassium

The loss of potassium of the post x-radiation series that received 600r followed the same general pattern as the series that received 700r (Graph 2). The pattern consisted of three phases: one, a marked increase in potassium excretion on day 0; two, an abrupt decrease to normal levels in the day 1, 7 and 14 series; and third, s sharp rise on day 30. The total loss for the series that received 500r or 600r was consistently higher than those that received the 700r doses.

(d) Post x-Radiation Characteristics of Syracuse Strain of Rats

(i) Mortality and Growth Rates (Table 1 and Graph 3)

A series of mortality and growth rate experiments were conducted to evaluate the biological effects of a single total body exposure of x-radiation at three different dose levels. The animals lost approximately 15 grams during the 18 hours pre-radiation fasting period. Food was restored immediately following x-radiation

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[2]Henceforth will denote the amount of electrolyte loss during the entire 180 minutes.

and by day 1 there was approximately a 4 gram body weight gain. This was followed by a slight weight loss on days 2 to 4, after which there was a small progressive weight increase to day 30 in those animals that survived. When there was a progressive daily weight loss the animals died. The percentage weight increase from the pre-radiation fasting period to day 30 for the 500r, 600r and 700r groups was 9.6, 4.3 and 4.8 percent respectively.

The percent survival for the radiated animals was as follows: day 14, at 600r and 700r, 71 and 90 percent survived respectively while at day 30 only 78 and 58 percent respectively survived. There were no deaths in the day 30, 500r group or in the non-radiated controls.

(ii) Hematocrits (Table 1)

The progressive decrease in the hematocrits through day 14 in all of radiation series that received 600r or 700r was followed by a slight increase.

(iii) Blood Pressures (Graph 4)

Systolic blood pressures were taken every second day for a period of thirty days on two series of rats that had received 600r or 700r. Although there were wide variations in some individual readings on any given day, each series followed a general depression-recovery pattern for each dose level. The majority of these individual variations were characterized by temporary elevated blood pressures of considerable magnitude, but subsequent readings were well within the average figures of their experimental group. It should be pointed out that the transient elevated pressures observed in the individual animals were probably due to manipulation procedures or excitability of the rat on a specific day.

The average blood pressures of both the x-radiated series of rats were characterized by two depression-recovery periods. Three days after radiation there was a precipitous fall in blood pressures, followed by a period of recovery. This first recovery period lasted from day 7 to 13 in the 600r series and from day 9 to 17 in the 700r. The second decline in systolic pressures continued to day 19 and day 23 in the 600r and 700r series respectively, after which there was a steady increase through day 30. The lowest pressures recorded were on day 23 of the animals exposed to 700r. Whenever the systolic pressures fell below 100 mm. Hg the animals died within 18 hours.

(2) Pregnant intact rats

(a) Diuretic Rates

The percentage of water excreted for each 30 minute period is shown in Table 4. These are generally lower than the diuretic rates found for non-radiated animals for the first 30 to 60 minutes after the second water dose. After the first hour most groups excrete about the same total percentage of water.

(b) Serum Electrolytes (Table 5)

In the pregnant radiated animals there is a tendency for the serum sodium to fall and then return to normal levels. The opposite holds for the non-pregnant radiated group where the tendency is for the serum sodium to increase and then return to normal. Insufficient figures are available for a complete statistical analysis of the serum sodiums, but the high values of the non-pregnant group are significantly different from the low values of the pregnant group ($p = .02$).

The serum potassium remains within normal limits in the non-pregnant radiated animals but in the pregnant group it is almost 200% of normal at 14 days (2 days post-radiation). It then gradually falls to normal levels by 18 days (6 days post-radiation). The high point of 11.1 at 14 days is the highest serum potassium seen to date in this laboratory.

No explanations can be offered for these serum sodium and potassium values.

(c) Urine Electrolytes (Table 5)

Sodium

In both the pregnant and non-pregnant groups there is evidence of a dumping of sodium into the urine toward the end of gestation. Examination of Table 5 shows that there is more than twice as much sodium in the urine of the pregnant as in the urine of non-pregnant animals on the 22nd day (10 days post-radiation). An increase in sodium excretion a few days prior to delivery is seen in normal pregnant animals.

Potassium

The potassium excreted by the pregnant radiated animals shows a progressive increase from the 14th day to the time of delivery. This increase is shown by the control animals up to the 20th day, but there is a drop in the potassium excreted on the day of delivery.

(d) Hematocrit (Table 4)

The decline in hematocrit values follows the pattern set by animals receiving 600r and 700r. This decrease is not repaired in the pregnant radiated animals even by the time of delivery (10 days post-radiation). In the controls, the hematocrit values reach their low point at 14 days (2 days post-radiation) and by 18 days (6 days post-radiation) they have returned to the normal values. The continuing lower readings for the pregnant radiated animals are probably the result of the anemia of pregnancy rather than of radiation damage. Bond ('48) has found that in pregnant rats the hematocrit is 40.0 on the 13th - 14th day and 38.6 on the 21st - 22nd day. His values agree well with those presented here.

(e) Fetuses

Fetuses were examined for gross malformations which might have resulted from exposure to x-rays. None were found in the 250r groups although malformations were found in fetuses from three pregnant females exposed to 500r.

That a dose of 250r has no effect on the embryos is unlikely. Of 76 embryos examined 8 days after radiation, 71% were judged to be viable while 29% were dead or resorbed. Ten days after radiation 41% of 49 embryos were alive and 59% were dead or resorbed. These percentages for dead or resorbed fetuses are in fair agreement with the figure of 46.4% given by Dr. James Wilson ('51) for fetuses examined after irradiation with 200r at ten days of pregnancy. The studies of Dr. Wilson are not strictly comparable with ours, for different methods and times of exposure have been used.

C. Conclusions

Our data support the following conclusions:

Non-pregnant animals

(1) no significant changes in the diuretic rates at the LD_{50} dose level indicate there is no damage to the kidney tubules.
(2) there is an apparent progressive dysfunction of the adrenal cortex (zona glomerulosa) at all 3 dose levels, as evidenced by the excessive electrolyte loss by the day 30 series.
(3) the serum sodium and potassium concentration levels remained within the limits of the non-radiated controls in spite of the high renal wastage.
(4) a revaluation is needed of the day 14, 600r and 700r series in that there was a complete restoration of electrolyte balance and blood pressures.
(5) a progressive decrease in hematocrits through day 14 was followed by a slight recovery.

Pregnant animals

(1) diuretic rates indicate no damage to kidney tubules.
(2) serum sodium and potassium values are displaced but return to normal by the time of delivery.
(3) urine electrolytes are higher following radiation with even 250r.
(4) the number of dead and resorbed embryos indicate that this dosage has a deleterious effect upon embryonic development.

It is apparent from our data that there is a sufficient quantity of cortical hormone available to the kidney tubule to maintain normal diuretic rates through day 30, but an insufficient amount to maintain electrolyte balance. Our present data does not lend support to the fact that a full blown adrenal cortical insufficiency was present.

It is suggested that the secretory tissues of the adrenal cortex exhibit different degrees of radiosensitivity.

(3) Publications

One publication ready for press.

(4) Other Information

(a) Analysis of present data indicated the need of the following changes in procedure.

(1) X-radiation doses above the LD_{50} (700r) level to the kidney *per se*.
(2) Increase the urine collection periods from 3 to 8 hours in both the non-pregnant and pregnant animals.

(b) No changes in personnel since 1 September 1952, activation date of contract.

(c) Graduate students on contract.

(1) One (1) full time research assistant.
(2) Two (2) technical assistants on hourly basis for electrolyte analysis, x-ray and blood pressure determinations.

(d) Two senior investigators, full time during summer.

(e) University furnishes the following support.

(1) One (1) full time animal caretaker.
(2) Secretarial help.

(f) No major difficulties encountered.

(5) Addendum

A supplementary report will be forth coming (1 Sept. 1953) of work in progress during July and August.

*TABLE I - Showing diuretic rates and hematocrit determinations for control and irradiated rats.

DAYS POST IRRADIATION	NUMBER OF ANIMALS	HEMATOCRIT	PER CENT WATER EXCRETED IN MINUTES 30	60	90	120	150	180	PERCENT SURVIVAL
Controls	12	43.4	0.0	12.5	44.1	69.2	81.3	87.1	100%
				Dose - 500r					
30 days	18	40.9	1.4	29.5	56.3	73.0	82.2	88.9	100%
				Dose - 600r					
Day of Radiation	12	43.3	0.2	36.1	64.9	74.4	79.4	85.6	100%
18 hrs.	12	42.9	5.8	36.0	67.3	77.0	81.4	89.5	100%
7 days	21	38.2	0.0	26.2	55.4	73.5	81.4	85.4	100%
14 days	12	36.0	0.0	5.9	38.4	65.2	78.6	86.0	71%
30 days	14	39.9	4.9	31.1	55.2	75.0	85.5	92.2	78%
				Dose - 700r					
Day of Radiation	12	43.4	1.4	31.5	60.6	72.4	74.9	80.8	100%
18 hrs.	12	42.3	4.1	34.2	63.1	73.2	81.1	87.7	100%
7 days	19	37.7	3.5	27.3	54.0	72.0	78.6	85.4	100%
14 days	18	34.5	0.2	19.8	52.5	72.5	84.9	87.5	90%
30 days	10	39.9	3.7	35.8	64.6	79.5	87.6	90.6	56%

*NOTE: Revised

Table 2
ELECTROLYTES

Sodium

DAYS POST IRRADIATION	SERUM	URINE				
		First 90 minute Collecting period		Second 90 minute Collecting period		
		mEq/l.	Total mEq	mEq/l.	Total mEq	Total mEq 0 - 180 min.
Controls	138.6	4.8	.030	6.6	.032	.062
D O S E - 500r						
30 days	146.2	16.5	.141	59.2	.204	.345
D O S E - 600r						
Day of Radiation	142.5	4.5	.038	27.3	.063	.101
18 hrs.	144.2	10.7	.094	19.7	.052	.146
7 days	143.6	14.7	.111	32.3	.078	.189
14 days	140.9	7.3	.038	5.8	.031	.069
30 days	146.2	9.5	.081	27.3	.112	.193
D O S E - 700r						
Day of Radiation	145.3	6.9	.057	26.0	.066	.123
18 hrs.	145.3	11.5	.097	18.1	.050	.147
7 days	142.4	12.5	.094	19.5	.045	.139
14 days	139.9	6.9	.045	6.6	.029	.074
30 days	146.7	12.3	.114	24.6	.075	.189

Table 3
ELECTROLYTES

Potassium

DAYS POST IRRADIATION	SERUM	URINE: First 90 minute Collecting period mEq/l.	URINE: First 90 minute Collecting period Total mEq	URINE: Second 90 minute Collecting period mEq/l.	URINE: Second 90 minute Collecting period Total mEq	Total mEq 0 - 180 min.
Controls	6.1	9.1	.052	8.0	.042	.094
D O S E - 500r						
30 days	6.2	12.3	.093	19.3	.064	.157
D O S E - 600r						
Day of Radiation	6.0	6.4	.051	36.1	.089	.140
18 hrs.	6.0	5.5	.050	18.0	.049	.099
7 days	5.2	9.9	.067	18.1	.047	.114
14 days	5.3	8.9	.047	6.9	.041	.088
30 days	6.0	10.6	.084	18.4	.081	.165
D O S E - 700r						
Day of Radiation	5.4	9.2	.069	37.9	.090	.159
18 hrs.	6.3	5.9	.049	12.7	.037	.086
7 days	5.2	8.3	.055	11.9	.032	.087
14 days	5.6	10.6	.063	8.6	.037	.100
30 days	5.8	7.5	.052	17.6	.064	.116

Table 4
DIURETIC RATES FOR FOUR GROUPS OF RATS AND HEMATOCRIT VALUES FOR RADIATED PREGNANT AND NON-PREGNANT RATS

DIURETIC RATES
PERCENT WATER EXCRETED IN MINUTES

Day of Pregnancy	Day Post Radiation	30 a	30 b	30 c	30 d	60 a	60 b	60 c	60 d	90 a	90 b	90 c	90 d	120 a	120 b	120 c	120 d	150 a	150 b	150 c	150 d	180 a	180 b	180 c	180 d
14	2	10	24	37	30	29	42	61	49	51	66	74	64	70	75	75	77	80	83	88	83	86	88	88	85
16	4	15	37	42	30	36	49	62	49	55	65	76	64	73	80	85	77	83	87	86	83	89	90	87	85
18	6	15	18	34	39	33	39	56	49	57	67	67	64	72	81	78	77	85	89	82	83	90	91	83	85
20	8	20	11	26	30	42	30	45	49	62	54	61	64	77	87	70	77	89	87	74	83	89	95	77	85
22	10	22	11	29	30	48	27	49	49	66	43	65	64	75	60	79	77	78	74	85	83	80	84	86	85

a--pregnant, radiated animals
b--non-pregnant, radiated animals
c--pregnant, non-radiated animals
d--non-pregnant, non-radiated animals

HEMATOCRITS

Day of Pregnancy	Day Post-Radiation	Pregnant, Radiated	Non-Pregnant, Radiated
14	2	40.1	38.5
16	4	37.1	37.8
18	6	38.8	43.8
20	8	38.0	42.4
22	10	39.5	42.7

Table 5
ELECTROLYTES

SODIUM

	Serum	Urine Tot. mEq.
Non-pregnant, non-radiated	140	.120

		Pregnant, Radiated		Non-pregnant, Radiated	
Day of Pregnancy	Days Post-Radiation	Serum	Urine Tot. mEq.	Serum	Urine Tot. mEq.
14	2	140.0	.066	----	----
16	4	138.2	.095	144.4	.093
18	6	136.1	.141	141.3	.132
20	8	138.2	.142	138.6	.245
22	10	142.3	.591	142.1	.204

POTASSIUM

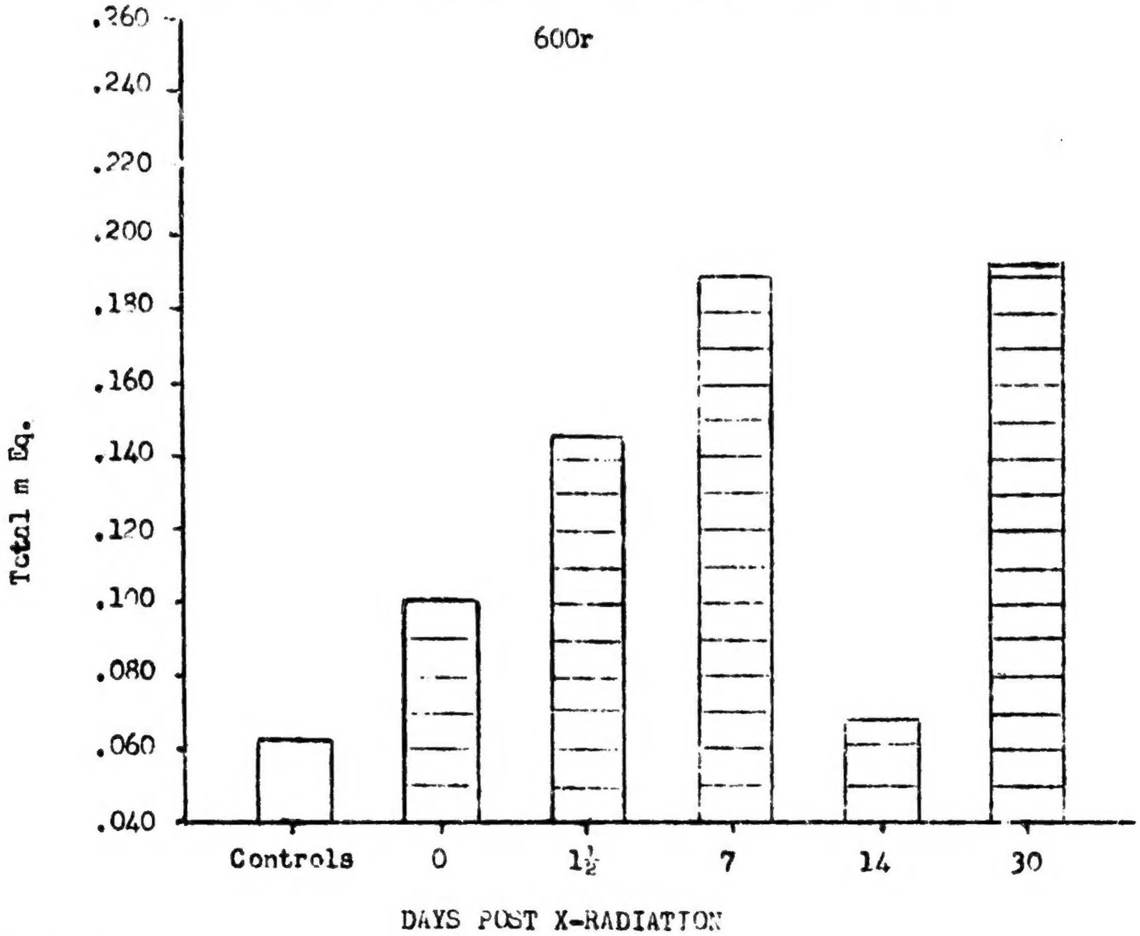
	Serum	Urine Tot. mEq.
Non-pregnant, non radiated	6.0	.134

		Pregnant, Radiated		Non-pregnant, Radiated	
Day of Pregnancy	Days Post-Radiation	Serum	Urine Tot. mEq.	Serum	Urine Tot. mEq.
14	2	11.1	.121	----	----
16	4	8.6	.132	6.79	.102
18	6	6.1	.148	6.6	.145
20	8	5.3	.160	5.4	.163
22	10	6.1	.243	5.7	.137

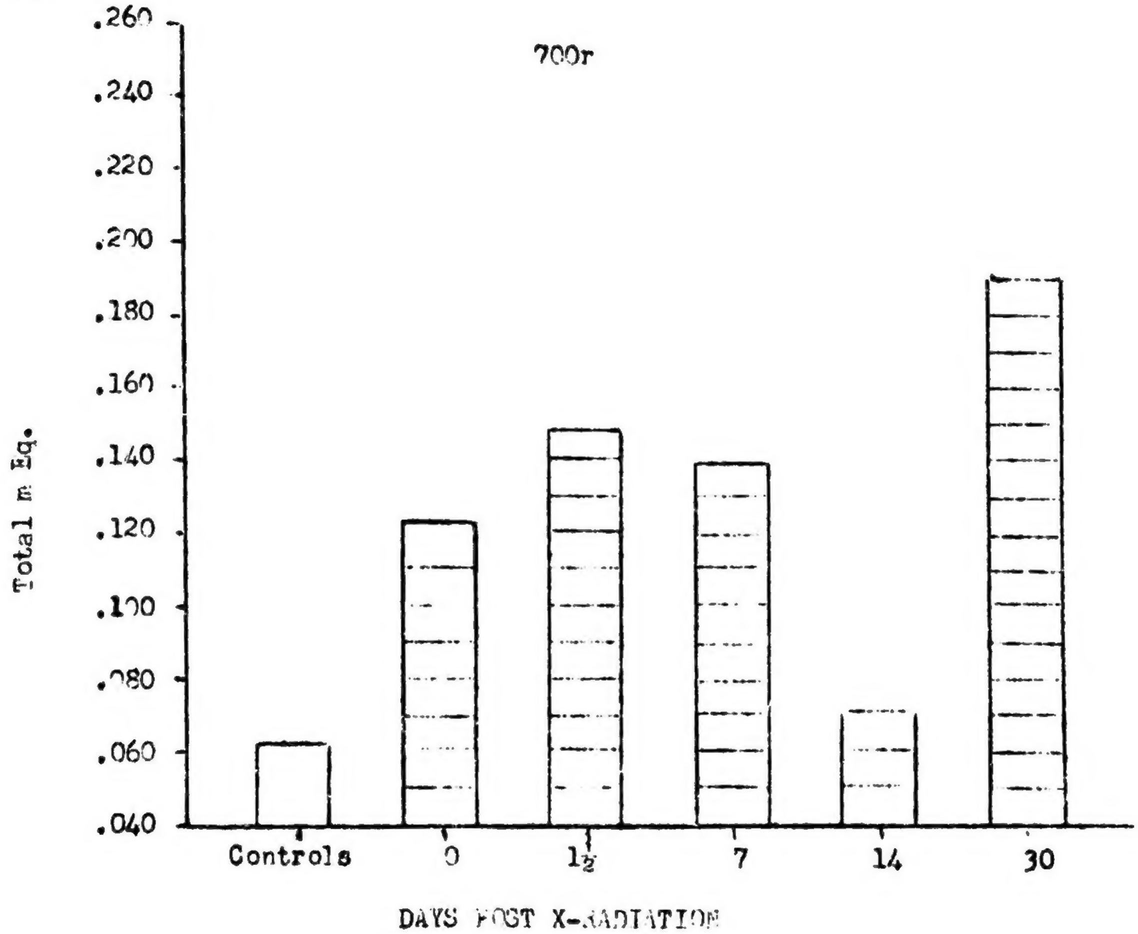
Graph 1

Sodium

Sum total m Eq. loss of sodium during 180 minutes

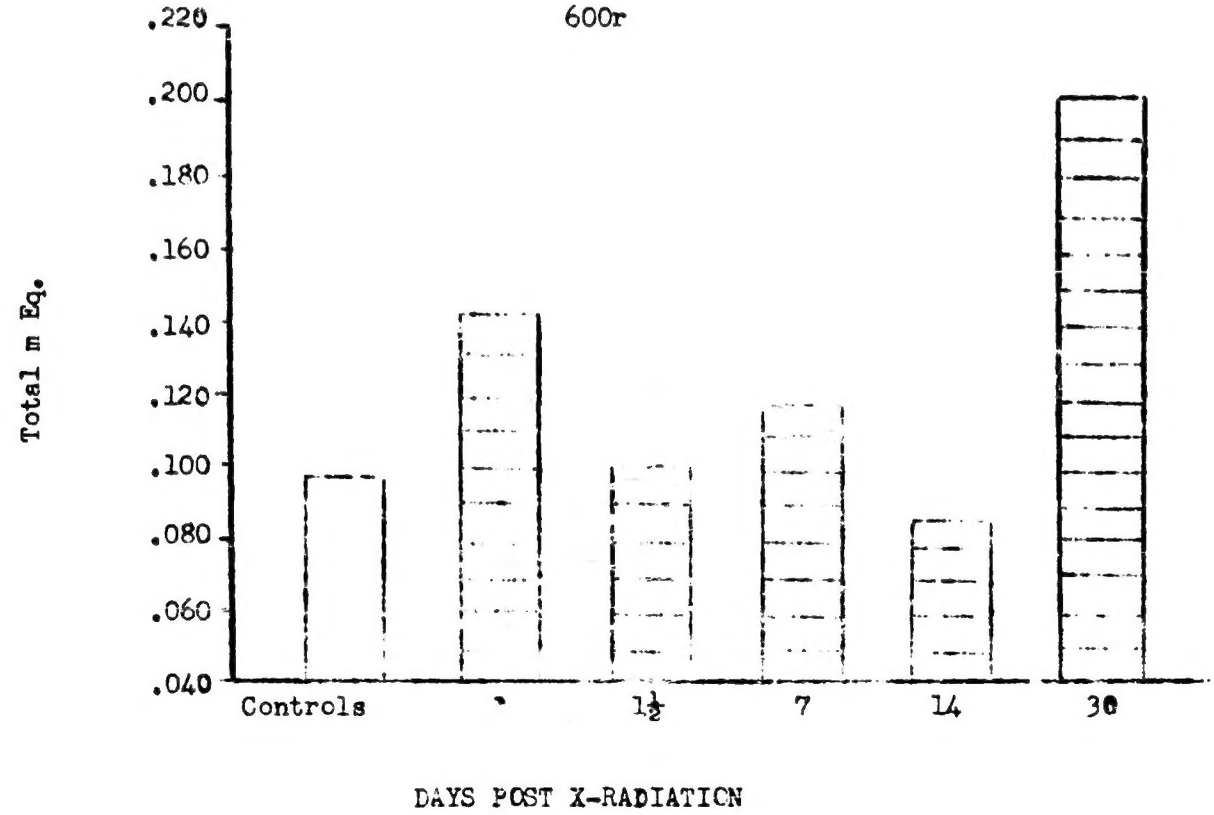


Graph 2

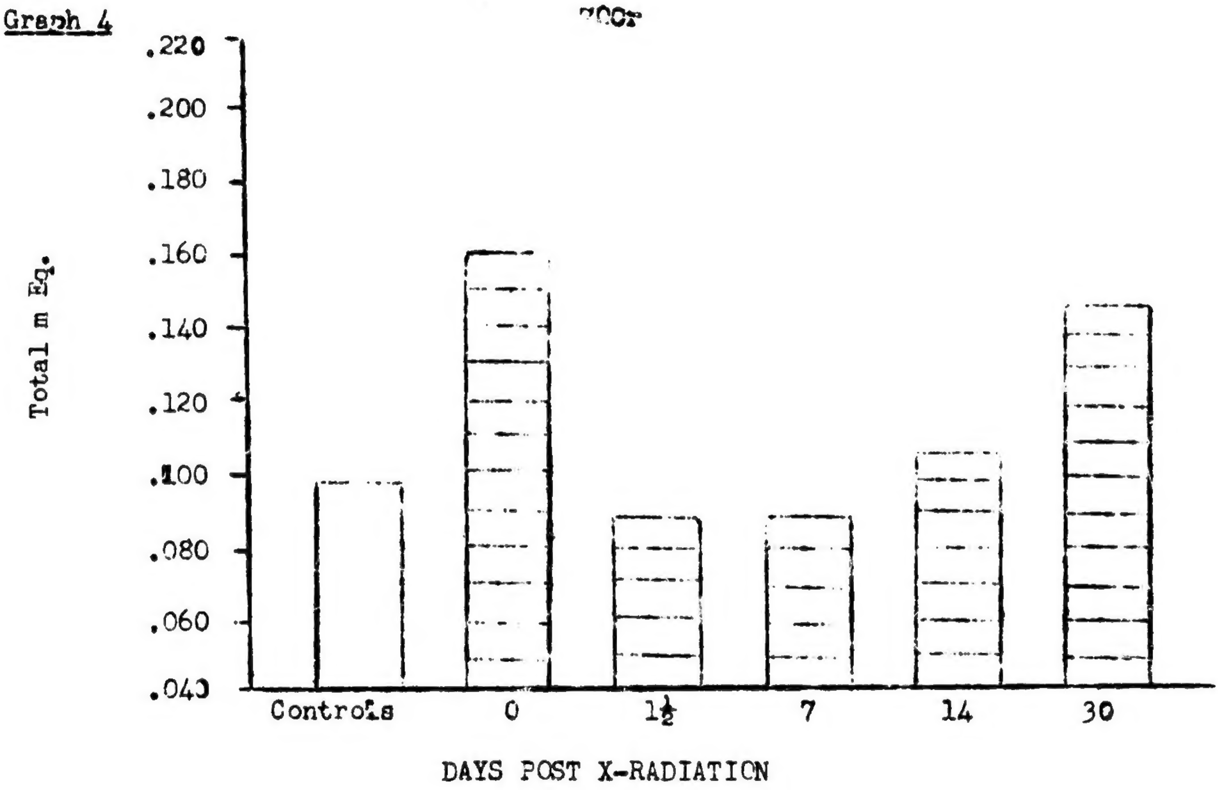


Potassium

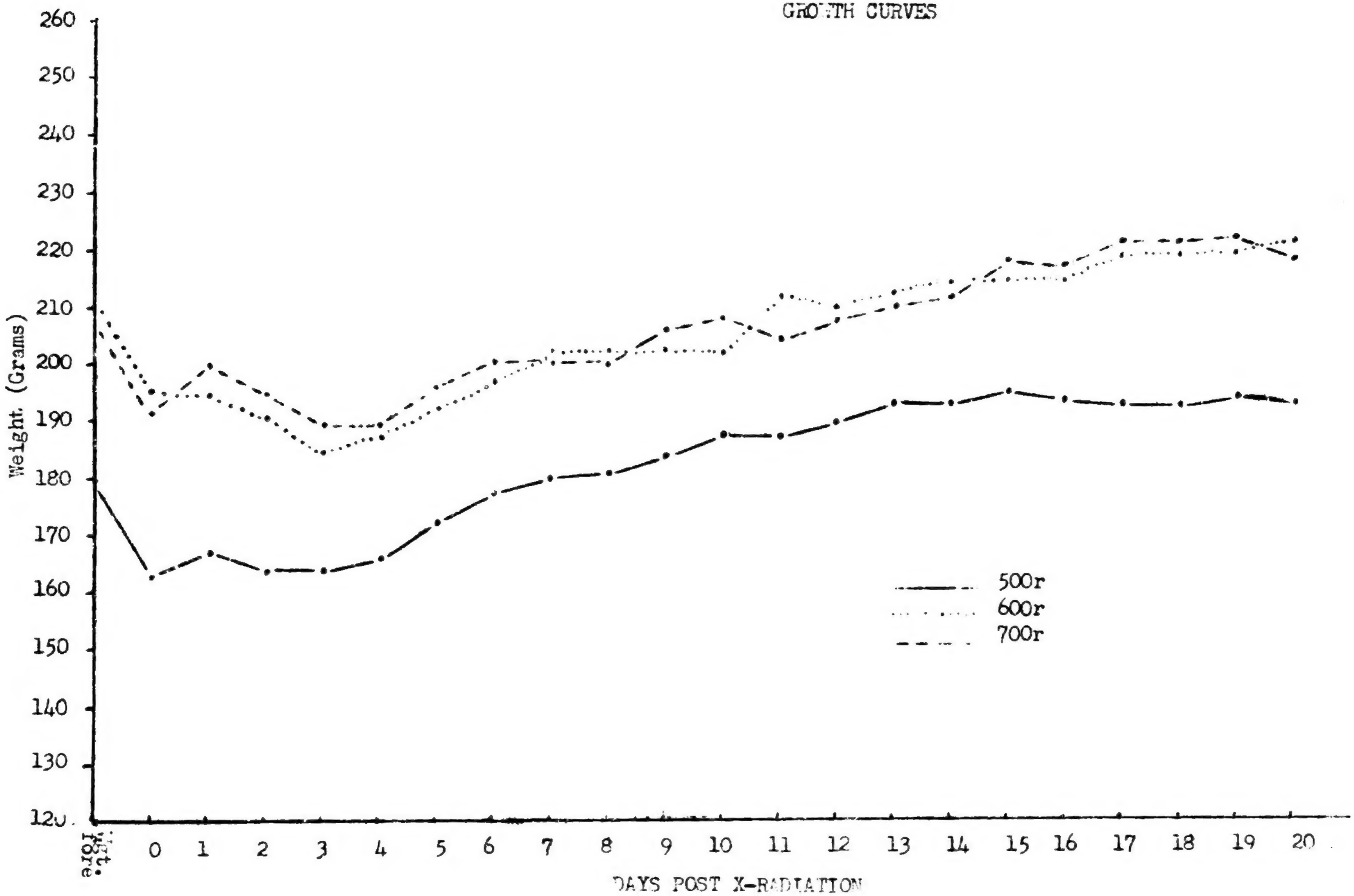
Graph 3 Sum total m Eq. loss of potassium during 180 minutes



Graph 4



GROWTH CURVES



Graph 6

BLOOD PRESSURE OF X-RADIATED ANIMALS